

THE MASS SPECTRA OF ω -AMINO ACID DERIVATIVES.

THE ROLE OF ω -ACTIVATION IN PRODUCTION OF IONS UPON ELECTRON-IMPACT*

S. OSMAN,† P. HOAGLAND and C. J. DOOLEY

Eastern Regional Research Laboratory,‡ 600 E. Mermaid Lane,
Philadelphia, Pennsylvania 19118, USA

Abstract—The mass spectra of a series of N-phthaloyl and N-trifluoroacetyl derivatives of ω -amino acids ranging from 3-aminopropionic acid to 6-aminohexanoic acid were determined. Ions of significant intensity resulting from the loss of neutral fragments from precursor ions were observed. Deuterium labeling studies indicate the initial fragmentation loss of a neutral molecule; i.e. the loss of water from the molecular ion involves ω -hydrogen loss from the alkyl chain. A fragmentation scheme consistent with metastable, high resolution and deuterium labeling data is presented.

A SERIES of ω -amino acid derivatives were recently prepared in this laboratory in an attempt to introduce amino groups of different basicities into proteins by amino acylation of lysyl ϵ -amino groups.¹ The anticipated need for identifying these compounds in hydrolysates of these proteins and the fact that they have been shown to have varied biological functions² prompted a systematic study of the mass spectral fragmentation of the ω -amino acid derivatives.

The spectra of ω -amino acid derivatives vary considerably from the much studied α -amino acid derivatives.³ For many of the derivatives of the ω -amino acids a series of ions arising from the loss of neutral species is observed. We have attempted to determine the path through which these ions are formed in order to rationalize the fragmentation of this class of compounds. The variation in the fragmentation pattern with change in the N-substituent as well as alkyl chain length will be explained based on a fragmentation path established by metastables, deuterium labeling data and high resolution data.

Results and discussion

The mass spectral fragmentation patterns of ω -N-trifluoroacetyl amino acids of increasing chain length are summarized in Table 1. Molecular ions have been tabulated although they are of negligible intensity.

The first significant fragment ion in all the spectra is $[M - 18]^+$. The possibility that this is the molecular ion resulting from thermal decomposition of the acid prior to ionization is unlikely, since appropriate metastables for the $[M]^+ \rightarrow [M - 18]^+$ transition are observed and variation of source temperatures had no noticeable effect on relative ion intensities. All spectra also have an intense ion at $[M - 46]^+$ which is

* Presented in part at the ASMS, 20th Annual Conference on Mass Spectrometry and Allied Topics, Dallas, Texas, June 1972.

† Author to whom inquiries should be addressed.

‡ Agricultural Research Service, Northeastern Region, US Department of Agriculture.

TABLE 1. MASS SPECTRA OF N-TRIFLUOROACETYL DERIVATIVES OF ω -AMINO ACIDS

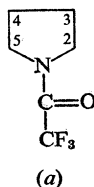
3-aminopropionic acid	m/e	185	167	140	139	126	115	98	78	70	69
	% I ^a	<0.1	10	5.0	24	24	7.0	14	2.9	5.0	7.4
4-aminobutanoic acid	m/e	199	182	181	153	140	139	126	112	98	69
	% I	<0.1	2.0	15	7.7	19	9.9	29	5.8	4.4	11
5-aminopentanoic acid	m/e	213	195	177	167	154	140	139	127	126	98
	% I	<0.1	18	4.2	4.2	3.5	4.6	14	3.9	35	4.9
	m/e	69									
	% I	8.8									
6-aminohexanoic acid	m/e	227	209	181	168	165	152	140	139	127	126
	% I	<0.1	4.5	2.8	5.9	12	2.0	8.7	1.4	5.3	28
	m/e	114	98	97	69						
	% I	5.3	2.5	5.3	14						

^a % I = Σ_{60}

derived, at least in part, from $[M - 18]^+$ as determined by metastable scanning.* High resolution data (HRMS) confirm these ions to be $[M - H_2O]^+$ and $[M - H_2O - CO]^+$. Other significant ions in the high mass end of the spectrum of these compounds also arise through the loss of neutral molecules. In order to rationalize a pathway for the formation of these ions, a series of deuterated 5-N-trifluoroacetylaminopentanoic acids was prepared. The mass spectra of these compounds are summarized in Table 2.

Only when C-5 is labeled do we observe a significant shift of $[M - 18]^+$ to $[M - 19]^+$. Based on this fact, a scheme for the formation of $[M - 18]^+$ and ions derived from $[M - 18]^+$ is shown in Scheme 1. Molecular formulae for the ions are based on HRMS data and each of the transitions is substantiated by an appropriate metastable. We have drawn structures for the ions only to provide a useful device for illustrating which atoms are lost in the fragmentation.

Although cyclic structures can be shown for m/e 195 and m/e 167, we have chosen to draw these as open chain structures for the following reasons. A pyrrolidine type structure (a) for m/e 167 suggests the equivalency of C-5 to C-2 (as well as C-3 to C-4) which should result in the equal expulsion of C-5 and C-2 in the loss of ethylene to form m/e 139. The spectra of the deuterium labelled compounds do not support the

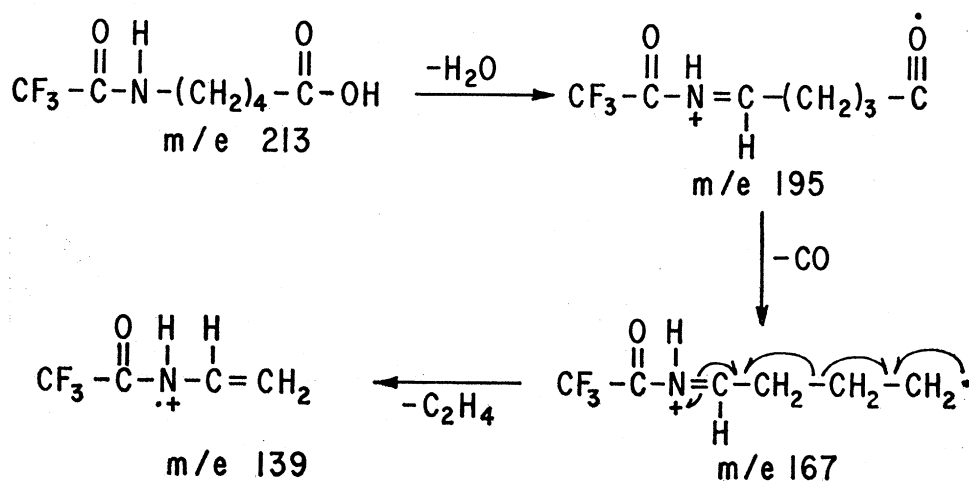


equivalency of C-5 and C-2. The data indicate that C-5 is retained (in 5,5 d_2 , m/e 139 is shifted to m/e 140) and C-2 is not retained in m/e 139. The data in Table 2 do suggest almost quantitative loss of C_2 and C_3 in this fragmentation step. An open

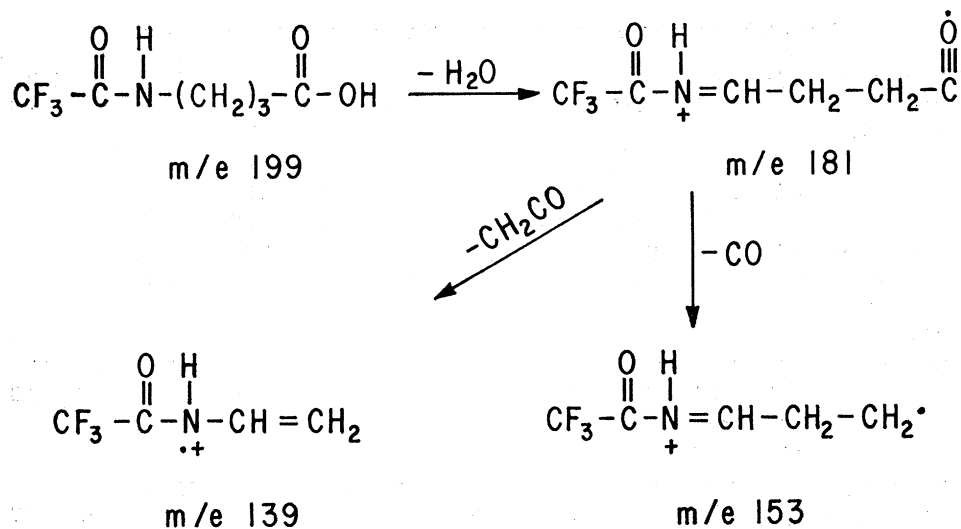
* Metastable data were obtained by varying the accelerator voltage in order to focus a chosen daughter ion derived from various parent ions.

TABLE 2. MASS SPECTRA OF DEUTERATED N-TRIFLUOROACETYLAMINO ACIDS

5-N-trifluoroacetylaminopentanoic acid	<i>m/e</i> % I ^a	198 3.7	197 3.7	196 10	178 5.5	169 3.7	168 3.7	157 3.7	154 1.8	153 1.8	142 1.8
5,5- <i>d</i> ₂ ^b	<i>m/e</i> % I	141 4.2	140 8.7	129 3.7	128 26	127 1.8	98 2.1	83 2.1	82 1.8	81 1.6	69 7.7
5-N-trifluoroacetylaminopentanoic acid	<i>m/e</i> % I	198 1.9	197 7.5	196 2.2	178 3.8	169 2.2	168 2.2	167 1.9	156 3.6	155 1.9	151 1.9
3,3- <i>d</i> ₂ ^c	<i>m/e</i> % I	140 3.8	139 10	128 2.2	127 2.2	126 28	98 3.8	84 1.9	83 2.5	78 3.8	70 3.8
	<i>m/e</i> % I	69 7.5									
5-N-trifluoroacetylaminopentanoic acid	<i>m/e</i> % I	198 2.3	197 7.2	179 5.4	169 3.1	154 2.7	153 2.3	140 5.4	139 11	128 2.7	127 2.7
2,2- <i>d</i> ₂ ^d	<i>m/e</i> % I	126 23	101 7.7	100 5.0	84 3.6	83 2.7	78 5.0	69 9.0			
3-N-trifluoroacetylaminopropionic acid	<i>m/e</i> % I	168 3.0	167 1.2	141 4.7	140 18	139 7.2	126 21	117 3.0	99 8.6	98 4.1	81 2.7
2- <i>d</i> ₁ ^e	<i>m/e</i> % I	78 3.7	71 7.6	69 15							

^a % I = Σ₉₀^b 85% D₂ determined from [M]⁺ of benzoyl derivative.^c 75% D₂ determined from [M]⁺ of phthaloyl derivative.^d >95% D₂ determined from [M]⁺ of underivatized amino acid.^e 63% D₁ determined from [M]⁺ of phthaloyl derivative.

SCHEME 1. Partial fragmentation pathway of 5-N-trifluoroacetylpentanoic acid on electron-impact.



SCHEME 2. Partial fragmentation pathway of 4-N-trifluoroacetylaminobutanoic acid.

chain structure seems most consistent with these results. (A coiled structure as suggested by Djerassi⁴ in the fragmentation of ether esters may be a reasonable alternative to the structures as we have illustrated them.)

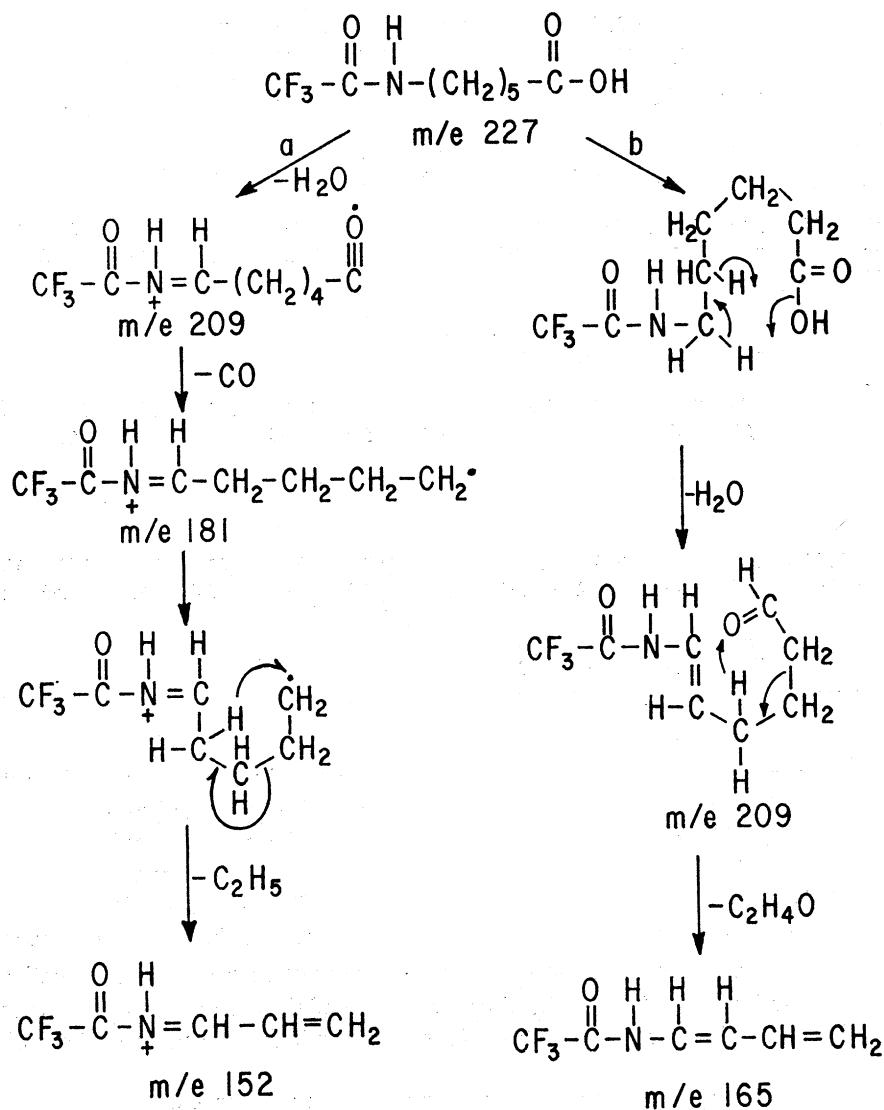
Similar fragmentation pathways can be drawn for the other compounds in this series. In Scheme 2 we show the fragmentation of 4-N-trifluoroacetylaminobutanoic acid. Again this pathway is supported by metastable and HRMS data. *M/e* 139 is a daughter ion of 181 as one would predict from this scheme. Consideration has been given to the possibility of *m/e* 139 arising from McLafferty rearrangement of the molecular ion. There are, however, no metastable ion data that support such a pathway. *M/e* 153 is depicted similarly to *m/e* 167 in Scheme 1 as a matter of consistency.

The fragmentation of 6-N-trifluoroacetylaminohexanoic acid is shown in Scheme 3. Pathway b is similar to that proposed by Meyerson and Leitch⁵ for the fragmentation of 6-phenylhexanoic acid. For both pathways a and b, appropriate metastables are observed.

The fragmentation of 3-N-trifluoroacetylaminopropionic acid is of interest because, in contrast to the other members of the series, $[\text{M} - \text{H}_2\text{CO}_2]^+$ is a daughter ion of both $[\text{M} - \text{H}_2\text{O}]^+$ and $[\text{M}]^+$. (Ballantine and Curtis⁶ have reported the loss of the elements of formic acid from the molecular ion of methyl 3-phenylpropionate as a one-step process.) Labeling studies have confirmed the 3 hydrogen as the alkyl hydrogen lost in these fragmentations.

Mass spectral data for the fragmentation of the phthaloyl derivatives of the ω -amino acids is given in Table 3. The fragmentation of these derivatives appears to be similar to the ω -trifluoroacetyl derivatives with respect to the formation of $[\text{M} - 18]^+$ and ions derived from $[\text{M} - 18]^+$. One noteworthy difference is the significant parent ions in the spectra of the phthaloyl derivatives. The spectra of phthalimido methyl esters* are comparable to the free acid spectra, i.e. ions formed by the loss of methanol and this ion in turn losing CO, C₂H₄, etc.

* These spectra will be submitted to Aldermaston data files.



SCHEME 3. Partial fragmentation pathway for 6-N-trifluoroacetylaminohexanoic acid.

The general nature of the fragmentary loss of neutral species in the ω -amino acid derivatives described above, appears to be quite analogous to the fragmentation of ω -phenylalkanoic acids described by Meyerson and Leitch. These workers theorize that the driving force for the $[\text{M}]^+ \rightarrow [\text{M} - 18]^+$ decomposition is the 'localized activation' of the phenyl substituent. Their data also suggest that the extent to which this fragmentation competes with other modes of fragmentation will, in part, be determined by the intermediate ring size, i.e. 4-phenylbutanoic acid can be pictured as losing water through a 6-membered ring intermediate; therefore $[\text{M}]^+ \rightarrow [\text{M} - 18]^+$ is a more important fragmentation path here than for the other members of the series.

TABLE 3. MASS SPECTRA OF ω -PHTHALIMIDO ACIDS

3-Phthalimido-propionic acid	<i>m/e</i>	219	202	201	174	173	161	160	146	145	133
	% I ^a	6.5	1.0	1.0	11	50	4.0	50	4.0	3.5	4.5
	<i>m/e</i>	130	105	104	103	102					
	% I	7.0	8.5	19	3.5	3.5					
4-Phthalimido-butanoic acid	<i>m/e</i>	233	215	214	188	187	175	174	173	161	160
	% I	6.0	10	3.0	1.7	4.7	3.0	25	14	5.6	4.3
	<i>m/e</i>	148	120	104							
	% I	2.2	2.2	2.6							
5-Phthalimido-pentanoic acid	<i>m/e</i>	247	230	229	201	188	174	173	161	160	130
	% I	6.0	2.4	10	2.8	5.6	4.8	11	8.0	40	8.0
	<i>m/e</i>	105	104								
	% I	2.8	2.8								
6-Phthalimido-hexanoic acid	<i>m/e</i>	261	243	215	202	199	174	161	160	148	134
	% I	4.3	4.2	2.2	2.6	6.5	2.6	6.0	43	9.0	2.2
	<i>m/e</i>	130	106	105							
	% I	5.2	3.0	3.4							

^a % I = Σ_{60}

Finally, the resultant ion can be stabilized by internal solvation. Our results are in agreement with those of Meyerson as to the effect of ring size on the $[M]^+ \rightarrow [M - 18]^+$ fragmentation. We have also obtained data as to the effect of substituents on the course of this fragmentation. In Table 4 we have listed the relative intensities of ions $[M]^+$, $[M - H_2O]^+$, $[M - H_2O - CO]^+$ and $[M - H_2O - CO - C_2H_4]^+$, ($[M - 18]^+$, $[M - 46]^+$, $[M - 74]^+$, respectively), as well as the ion formed by simple cleavage β to the nitrogen ($[N - C_{\frac{3}{2}}C]^+$) for a variety of N-substituted derivatives.* Where intense ions are observed by simple cleavage of the carbon to carbon bond at the carbon α to the nitrogen function, ions that result from cleavage of a carbon

TABLE 4. SELECTED PEAK INTENSITIES FROM THE SPECTRA OF Δ -AMINOVALERIC ACID DERIVATIVES

Derivative	$[M]^+$	$[M - 18]^+$	$[M - 46]^+$	$[M - 74]^+$	$[N - C_{\frac{3}{2}}C]^+$
TFA	—	50	15	40	100
Phthaloyl	30	30	8	30	100
Acetyl	6	2	2	5	20
Benzoyl	25	—	—	—	<1

* Wolff⁸ and co-workers have found $[M - CH_2OH]^+$ ions in the spectra of amino acid methyl esters. They have shown that the hydrogen comes from both the free amino group and the ω -carbon in the chain. Deuterium labeling of the nitrogen in the trifluoroacetyl derivatives indicates none or little N-hydrogen loss from this position in the formation of $[M - H_2O]^+$ and in the phthaloyl derivatives no such possibility exists.

hydrogen bond at the same carbon are also found in abundance. In the mono-functional amine derivatives, however, one does not usually find abundant $[M - 1]^+$ ions. In our system the fact that hydrogen is lost as part of neutral water more likely facilitates the carbon-hydrogen cleavage process. We have no explanation at this time for the apparent difference in the extent of β -cleavage between the derivatives. We are also interested in determining whether other functional groups can be substituted for the carboxyl function.

EXPERIMENTAL

Mass spectra were obtained on a Du Pont Model 492 mass spectrometer.* Batch inlet and source temperatures were approximately 200°C for all sample analyses. When possible samples were introduced via the direct probe. Low resolution data were obtained at 1000 resolving power, high resolution data at *ca.* 15,000.

Phthaloyl derivatives. The phthaloyl derivatives were prepared by a fusion of the amino acid and phthalic anhydride.^{9,10,11}

N-Trifluoroacetyl derivatives. Amino acids were trifluoroacetylated with trifluoroacetic anhydride or with ethyl thiotrifluoroacetate.^{12,13}

Melting points for all derivatives are listed in Table 5. Analytical data for new derivatives are also reported.

TABLE 5. MELTING POINTS AND ANALYSES OF ω -AMINO ACID DERIVATIVES

Compound	m.p. found	m.p. lit.	Theoretical			Found		
			C	H	N	C	H	N
phthaloyl glycine	196 to 198	192 to 194 ^a						
phthaloyl β -alanine	150 to 152	150 to 151 ^b						
phthaloyl γ -aminobutyric acid	115 to 117	113 to 115 ^c						
phthaloyl δ -aminovaleric acid	119 to 120		63.16	5.26	5.67	63.21	5.54	5.52
phthaloyl ξ -aminocaproic acid	107 to 108	107 ^d						
TFA glycine	116 to 118	117 to 119 ^e						
TFA β -alanine	119 to 121		32.44	3.27	7.57	32.66	3.63	7.52
TFA γ -aminobutyric acid	81 to 82		36.19	4.05	7.03	35.97	4.00	6.83
TFA δ -aminovaleric acid	94 to 95.5		39.44	4.69	6.57	39.67	5.81	6.34
TFA ξ -aminocaproic acid	89 to 90.5	88.6 to 90.6 ^f	42.30	5.32	6.17	42.13	5.14	6.17

^a J. H. Billman and W. F. Harting, *J. Amer. Chem. Soc.* **70**, 1473 (1948). ^b A. Schöberl and H. Braun, *Ann. Chem.* **542**, 274 (1939). ^c E. Späth, F. Kuffner and N. Platzer, *Ber. Deut. Chem. Ges. (B)* **68**, 699 (1935). ^d N. Z. Stanačev and M. Proštenik, *Arhiv Kem.* **27**, 203 (1955). ^e F. Weygand and R. Geiger, *Chem. Ber.* **89**, 647 (1956). ^f E. E. Shallenberger and M. Calvin, *J. Amer. Chem. Soc.* **77**, 2779 (1955).

5-N-Trifluoroacetylaminopentanoic acid 2,2-d₂. The sodium salt of 5-aminopentanoic acid (1 g) was dissolved in 10 ml of D₂O and about 2 mg of sodium was added.¹⁴ The solution was sealed in a Carius tube and heated in a 150° oven for 45 hrs. The solution was then concentrated and taken up in 10 ml of D₂O and reheated for 24 hrs. After concentration the residue was diluted with H₂O and the pH adjusted to 6.9. The solution was repeatedly concentrated (3×) with H₂O to remove deuterium from the amino and carboxyl groups. Mass spectra indicated more than 95% deuteration. The N-trifluoroacetyl derivative was made with ethyl thiotrifluoroacetate.¹³

5-N-Trifluoroacetylaminopentanoic acid 3,3-d₂. Thionyl chloride (4.5 g) was added to 3-phthalimidopropionic acid (3.5 g) in benzene (30 ml) and the solution was then refluxed for 2 hrs. The solution was concentrated under vacuum and the residue recrystallized from benzene-pet. ether.

The acid chloride (1.6 g) was added to a solution of sodium borodeuteride (0.2 g) in 10 ml of redistilled dioxane and refluxed overnight. The reaction was cooled, diluted with an equal amount

* The mention of commercial items is for convenience and does not constitute an endorsement by the Dept. of Agriculture.

of D₂O and extracted with benzene. The benzene extracts were dried over anhydrous sodium sulfate, filtered and diluted with an equal amount of pet. ether. The mass spectrum of the precipitate was consistent with the structure of 3-phthalimidopropanol 1,1-*d*₂OD.

The alcohol was converted to the bromide using PBr₃. The bromide was then added to a solution containing a molar excess of sodium diethyl malonate in 20 ml of absolute ethanol. After addition was complete the solution was refluxed for 1 hr., then cooled, neutralized with 10% H₂SO₄ and filtered. The filtrate was evaporated and the residue recrystallized from pet. ether. A portion was hydrolyzed in 10 ml of 5N NaOH. After acidification with HCl the amino acid hydrochloride solution was subjected to trifluoroacetylation.¹³

5-N-Trifluoroacetylaminopentanoic acid 5,5-d₂. To a solution of 5 g of 5-hydroxyvaleramide in 125 ml of freshly distilled THF, 4.0 g of LiAlD₄ was added over a period of 10 mins while cooling the reaction mixture in an ice bath. After addition was complete, the solution was allowed to warm to room temperature and then after 1 hr refluxed overnight. The solution was then treated with 50/50 MeOD/D₂O until decomposition was complete. The solution was adjusted to pH 2 with 1N HCl, then filtered and concentrated. The mass spectrum of the residue was consistent with 5-aminopentanol-5,5-*d*₂.

The amino alcohol was converted to the benzamide derivative by standard Schotten-Baumann procedures. The benzamide alcohol was oxidized to the benzamide acid derivative using alkaline permanganate. The compound, after recrystallization from benzene, yielded a mass spectrum consistent with 5-N-benzoylaminopentanoic acid 5,5-*d*₂. The compound was subjected to acid hydrolysis by refluxing in 1N HCl for 25 hrs. The N-trifluoroacetyl derivative was prepared directly from this solution with ethyl thiotrifluoroacetate.¹³

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